also be involved in the physiological and pathological process of articular cartilage.
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PHASE I CLINICAL TRIAL OF INTRA-ARTICULAR INJECTION OF AUTOLOGOUS MESENCHYMAL STEM CELLS FOR THE TREATMENT OF WRIST CHONDRAL DEFECT

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Introduction: Wrist chondral defect is a common cause of persistent joint pain, which may lead to functional impairments including reduced range of motion and diminished grip strength, affecting working ability and quality of life. There are various reported surgical treatment regimens but their effectiveness remains controversial due to the inherently poor regeneration ability of cartilage. Bone marrow-derived mesenchymal stem cells (MSC) are reported extensively to promote the regeneration of articular cartilage in various chondral defect and osteoarthritis animal models. While translation studies are on-going in knee OA, it is of clinical interest to explore the potential effectiveness of MSC therapy for the treatment of wrist chondral defect. As the first local MSC trial for chondral defect, we aimed to examine the feasibility and the safety, and to obtain data for sample size estimation for future study.
Subjects and methods: In this phase I single-arm trial, 10 patients (18 to 75 years old) with persistent post-traumatic chronic wrist pain, with imaging and previous arthroscopic evidence of wrist carpal bone chondral defects who opted for active treatment were invited to join (CREC Ref No. 2014.291-T). After arthroscopic washout and debridement, 10mL bone marrow was aspirated and subjected to the isolation and expansion of MSC cells in a certified clean room (ISO class 7). The MSC was characterized with a colony forming unit (CFU) assay, surface phenotypes (CD45, CD14, CD19, CD34, CD73, CD105, CD44, CD29, CD90, and HLA-DR), and a multipotent differentiation assay according to the International Society for Cellular Therapy guidelines. One month after bone marrow aspiration, characterised autologous MSCs (1 million cell per mL saline) with viability over 90% and clean from microbiological tests were injected back into the wrist joint of the patient. One-year follow-up assessments including functional wrist performance score and pain score, and other secondary assessments were carried out by trained personnel. The spread of data was tested for normality. Changes from baseline (pre-op) in all the measurements were determined with a t-test or Wilcoxon sign rank test where appropriate. Missing data was replaced with imputation under a missing-at-random assumption. Differences were considered statistically significant when $p < 0.05$ (SPSS V19).
Results: Six patients (5 male and 1 female), mean age of 38.5 years old fulfilling the inclusion and exclusion criteria were recruited during the reported period (January to December 2015). All procedures, including bone marrow aspiration, arthroscopic debridement, and intra-articular MSC injection, were uneventful and there were no signs of infection and nil complications noted or reported. It was practical to expand MSCs *in vitro* to a sufficient number for characterisation and injection in one month. Till now, six-month follow up data indicated the potential therapeutic effect of intra-articular MSC injection at single dose, as shown by numerical improvement in performance score and pain score.
Discussion and conclusion: This pilot clinical trial shows the safety and potential therapeutic effect of single dose autologous bone marrow-derived MSCs on persistent wrist chondral defect. Additional data from the second phase follow-up will provide more insight into the treatment of wrist chondral defect with MSCs.
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OPTIMISATION OF CULTURE CONDITIONS FOR MAINTAINING PORCINE INDUCED PLURIPOTENT STEM CELLS

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Ground state porcine induced pluripotent stem cells (piPSCs), which retain the potential to generate chimeric animal and germline transmission, are difficult to produce. This study investigated morphological and biological progression at the early stage of porcine somatic cell reprogramming and explored suitable conditions to increase the induction efficiency of piPSCs. A cocktail of defined transcription factors was used to generate piPSCs. The amphotropic retrovirus, which carried human OCT4 (O), SOX2 (S), KLF4 (K), C-MYC (M), TERT (T), and GFP were used to infect porcine embryonic fibroblasts (PEFs). The number of clones derived from OSKM (4F) and OSKMT (4F + T) was significantly higher than that from SKM (3F) and SKMT (3F + T), suggesting that OCT4 played a critical role in regulating porcine cell reprogramming. The number of alkaline phosphatase positive clones from a medium with leukaemia inhibitory factor (LIF) and basic fibroblast growth factor (bFGF) (M1 medium) was significantly higher than that with insulin and 2i PD0325901/CHIR99021 (M2 medium), indicating that insulin and 2i could not effectively maintain piPSC propagation. In the M1 medium, piPSC lines could not maintain the typical self-renewal morphology on gelatin-coated and Matrigel-coated plates. Without the mouse embryonic fibroblast (MEF) feeder, piPSCs started to simultaneously differentiate. Based on the potential for self-renewal and activation of pluripotent markers, we found that the culture condition of 4F + T plus LIF and bFGF plus MEF feeder promoted PEF reprogramming more efficiently than the other conditions tested. Two piPSC lines (IB-1 and IB-2) were derived and maintained for up to 20 passages *in vitro*.
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INJECTABLE AND ROBUST BIOPOLYMER-BASED SUPRAMOLECULAR HYDROGELS FOR REGENERATIVE MEDICINE

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Osteoarthritis (OA), which is symptomised as progressive degradation of articular cartilage in human diarthrodial joints, has become one of most prevalent, debilitating diseases in modern society. To address the increasing clinical demand for more effective treatment of OA, significant progress has been made in biotechnology, especially in the field of biomaterials. In the most recent decade, increasing research emphasis has been placed on the "bio" part of biomaterials. In our lab, we have shown that functionalisation of the hydrogels with biomimetic peptides promotes the differentiation of the hMSCs. In addition to the biofunctionalisation, the physical functions of the biomaterials are also critical to the successful translation of biomaterials to clinical treatment of cartilage diseases. Although biopolymer-based chemical hydrogels, with biopolymers covalently crosslinked, have been widely used as scaffolds for tissue engineering due to good stability, their permanent network structures and brittleness limit their applications in repairing load-bearing tissues, such as cartilage. In contrast, biopolymer-based supramolecular hydrogels, which are usually formed via self-assembly of physically interacting biopolymers are usually weak, as shown in "inverted vials", instead of freestanding 3D constructs and they are less stable than chemical hydrogels. Herein, we describe a novel host-guest macromer (HGM) approach for preparation of biopolymer-based freestanding supramolecular hydrogels. We have developed a series of injectable hydrogels with unique properties such as resilient mechanical property, bioadhesiveness, injectability, and promoting recruitment of endogenous cells that are desirable for potential clinical applications in the regeneration of soft musculoskeletal tissues such as cartilage, meniscus, and intervertebral discs.
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CO-CULTURE OF HUMAN SYNOVIUM-DERIVED STEM CELLS AND CHONDROCYTES REDUCES HYPERTROPHY

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Introduction: Mesenchymal stem cells (MSCs) have emerged as a clinically relevant cell source for regenerative medicine, especially for cartilage repair; however, it still remains a challenge to recapitulate the functional properties of native articular cartilage using only MSCs. *In vitro* expansion of chondrocytes causes dedifferentiation. Lately, co-cultures of chondrocytes and bone marrow MSCs demonstrated enhanced functional properties of engineered cartilage. In this study, we aimed to assess the effect of co-culture of synovium-derived stem cells (SDSCs) and chondrocytes on *in vitro* chondrogenesis in serum-free TGF-β supplemented medium.
Methods: Isolation and expansion of cells: Human SDSCs and chondrocytes were isolated by sequential digestion from explants of total knee arthroplasty patients and incubated at 37 °C, 95 % humidity, and 5 % CO_2 in standard culture medium. We used passage 2 cells hereafter.
In vitro differentiation of cells: Expanded chondrocytes, SDSCs, and chondrocyte/SDSCs (5×10^5cells per pellet; co-culture ratio, 1:1) were cultured in chondrogenic medium for 1, 7, 14, and 21 days. The SDSC and chondrocyte/SDSC mixed pellets were cultured with the supplementation of 10 ng/mL of TGF-β1 and the chondrocyte pellets were cultured in chondrogenic medium only. The medium was changed twice per week.

Determination of glycosaminoglycan (GAG) content: The synthesised GAG was determined by binding to DMB dye and the total amount of GAG was normalised to the amount of DNA by a PicoGreen dsDNA assay.
mRNA expression assay: Expressions of type I, II, X collagen, SOX-9, and aggrecan were analysed by real-time PCR. Expression of GAPDH was taken as an internal control.
Histologic and immunohistochemical examination: Pellets from each time point were stained with H&E and safranin-O for proteoglycan detection. The expression of type II collagen was detected by immunohistochemical (IHC) staining.
Results: Co-culture of human SDSCs and chondrocytes exhibited significantly higher GAG synthesis and type II collagen content. Furthermore, expression of type X collagen, a marker of MSC hypertrophy, was significantly lower in the co-culture pellets. The co-culture groups exhibited up-regulation of type II collagen and Sox-9 at seven days, and type I collagen, type X collagen, and aggrecan at 14 days. Safranin-O staining and IHC staining for type II collagen showed that chondrogenic differentiation of the co-culture group were higher than those of the chondrocytes or SDSCs single culture groups.
Discussion: Our results demonstrated that mixed co-culture of SDSCs and chondrocytes reduced cellular hypertrophy and enhance *in vitro* chondrogenesis when compared to the culture of chondrocytes or SDSCs alone.
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THE EFFECT OF LEUKOCYTE DEPLETION IN PRP ON THE PROLIFERATION AND CHONDROGENESIS OF SYNOVIUM-DERIVED MSCs AND EXPANDED CHONDROCYTES

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Introduction: Platelet-rich plasma (PRP) has been advocated as one treatment strategy for cartilage tissue regeneration. To date, several different platelet-rich formulations have been made available, but a deep knowledge of their composition and mechanism of action in a specific clinical use is needed. There are many variations in PRP formulations, with both platelet and leukocyte concentrations having been identified as major constituents affecting the inflammatory responses after PRP injection. The aim of this study was to investigate the effect of leukocyte depletion in PRP on the proliferation and chondrogenesis of synovium-derived MSCs (SD-MSCs) and chondrocytes.
Methods: Preparation of PRP formulations: From donated human fresh blood, we prepared three formulations of PRP: (1) platelet-poor plasma (PPP), (2) PRP with very few leukocytes (P-PRP), and (3) PRP with high concentrations of both platelets and leukocytes (L-PRP). PPP is the upper layer of plasma after centrifuging 10cc whole blood at 2400rpm for 10 minutes. After 2nd centrifuging at 3600rpm for 15 minutes, P-PRP is the lower layer of plasma without buffy coat. L-PRP is the lower layer of plasma with buffy coat.
Cell culture and *in-vitro* expansion: SD-MSCs and chondrocytes were obtained from patients undergoing total knee arthroplasty. The primary cells were expanded in DMEM with the three formulations. The cell proliferation was measured using the MTT assay.
Chondrogenesis: Chondrogenic differentiation of SD-MSCs and expanded chondrocytes were induced using a high-density pellet culture system. Aliquots of 5×10^5 cells were centrifuged at 500*g* for 5 minutes, which were subsequently cultured for three weeks. The pellets of SD-MSCs and chondrocytes were cultured in chemically defined chondrogenic medium with 10 ng/ml of TGF-β1 and/or 100 ng/ml of BMP-2.
Real-time PCR: Total RNA from SD-MSCs and chondrocytes were extracted by using the RNeasy mini kit (Qiagen). Expressions of type II collagen, type X collagen, Aggrecan, and Sox9 were analysed by RT-PCR. Expression of GAPDH was taken as the internal control.
Histological and immunohistochemical examination: Pellets were stained with Safranin-O for proteoglycan detection. The expression of type II collagen was detected by IHC staining and was observed under microscopy.
Results: Leukocyte depletion also lowered the count of platelets. L-PRP showed more proliferative effect on SD-MSCs and chondrocytes than P-PRP. RT-PCR revealed higher cartilage gene expression in TGF-β1 induced chondrogenesis of SD-MSCs and chondrocytes with P-PRP. However, there was a negative effect of both formulations in TGF-β1/BMP induced chondrogenesis. In TGF-β1 and/or BMP induced chondrogenesis, the SD-MSC pellet with P-PRP showed more proteoglycan production than those with L-PRP. Type II collagen synthesis in the chondrocyte pellets with P-PRP was greater than the pellets with L-PRP. However, type II collagen synthesis in SD-MSCs pellets was not detected.
Discussion: Although leukocyte depletion in PRP showed a negative effect on proliferation of SD-MSCs and chondrocytes and a positive effect on the mRNA expression of the chondrogenic differentiation marker genes, it could not change the negative effects of PRP on chondrogenic differentiation of SD-MSCs.
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DIFFERENCES IN MORPHOLOGY, PROLIFERATION, DIFFERENTIATION AND IMMUNE PROFILE AMONG SINGLE-CELL CLONED STEM CELLS FROM THE SAME MESENCHYMAL STEM CELL ORIGIN

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Introduction: Mesenchymal stem cells (MSCs) are believed to be immune-privileged due to lack of antigen-presenting-cell related markers; however, evidence suggests that MSCs are immunogenic and are attacked by the immune system. Our research investigates the hypothesis that there are differences between MSC clones from the same individual in terms of their morphology, proliferation, differentiation, and immune profile. Our goal is to discover immune-privileged stem cells, which can act as a universal allogenic mesenchymal stem cell donor to facilitate bone ingrowth for osteosarcoma patients' status post tumour excision and prosthesis implantation.
Subjects and Methods: Serial dilutions of bone-marrow derived (BMMSCs) and adipose derived mesenchymal stem cells (ADMSCs) from the same animal were carried out in order to isolate single-cell clones. From a single animal we obtained three clones from BMMSCs and three from ADMSCs. This procedure was repeated for another two animals. The proliferation rate and cell doubling time of each clonal culture was measured. The proliferation rate of mixed clonal cultures was also measured. The tri-differentiation potential of the clonal cultures was compared and a comparison was also made with the original isolates from bone marrow and fat. The immune-privileged properties were measured by flow cytometry and immuno-staining for the major histocompatibility complex (MHC) antigens. To measure the immune response a mixed leucocyte reaction was used but where leucocytes from a different individual were mixed with the clonal MSC cells.
Results: All isolates were able to differentiate into osteoblasts, chondrocytes, and adipocytes. All clonal cultures revealed significantly different proliferation rates and doubling times when compared with each other and with mixed cultures. All clonal cultures showed different surface marker presentations, which included differences in the expression of MHC antigens. One clone isolated from ADMSCs showed a lack of MHCI and MHCII. Our mixed leucocyte reaction and MHC staining showed a variety of immune-modulation and this was related to the expression of the MHC antigens.
Discussion and Conclusion: All clones tri-differentiated and therefore show a degree of 'stemness'. MSCs are generally believed not to express MHCII and to be immune-privileged. However, this study shows that the expression of these antigens in clones isolated from bone marrow and from fat is variable. A heterogeneous result indicates individual differences between MSCs, even from the same origin. The immune response elicited by MSCs is complicated. MSCs have been shown to release interleukin 10, which could inhibit the immune response but on the other hand interferon-gamma could enhance MHCII presentation in some MSCs. Our results confirmed our hypothesis because clonal cultures isolated from different sources of MSCs in the same animal show differences in proliferation rate, morphology, and surface marker presentation. Mesenchymal stem cells are not immunogenic or immune-privileged. Individual differences highlighted through single-cell clonal cultures may be the key to finding universal immune-privileged MSCs for allogeneic transplantation.
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IRON SUCROSE (VENOFER®) LABELLED ADIPOSE DERIVED STEM CELL BY USING 3T MAGNETIC RESONANCE IMAGING TRACKING: *IN VITRO* STUDY

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Background: Adipose derived stem cells (ASCs) based therapy is a promising strategy to promote in musculoskeletal repair. Cell differentiation ability, trophic factor and immunomodulation are considered as possible mechanisms to regenerate new tissue. However, the cell fate after implantation is required to understand treatment effects. In this study, we would like to investigate Iron sucrose labeling as the tool for MSC fate. Cell immunophenotypes, cell viability, cell differentiation ability after labelling are evaluated. Iron sucrose labelled MSCs were determined using 3 tesla (3T) MRI *in vitro*
Materials and methods: Adipose-derived mesenchymal stem cells (ASCs) were isolated from liposuction specimen from subcutaneous tissue. Passage 3rd ASCs were labelled with iron sucrose by adding in basal culture media (1 mg/ml) and